Effects of Metformin Therapy on Coronary Endothelial Dysfunction in Patients With Prediabetes With Stable Angina and Nonobstructive Coronary Artery Stenosis: The CODYCE Multicenter Prospective Study

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Diabetes Care 2019;42:1946–1955 | https://doi.org/10.2337/dc18-2356

OBJECTIVE

To evaluate the effect of metformin therapy on coronary endothelial function and major adverse cardiac events (MACE) in patients with prediabetes with stable angina and nonobstructive coronary stenosis (NOCS).

RESEARCH DESIGN AND METHODS

Metformin therapy may be needed to reduce coronary heart disease risk in patients with prediabetes. A total of 258 propensity score–matched (PSM) patients with stable angina undergoing coronary angiography were enrolled in the study. Data from 86 PSM subjects with normoglycemia (NG), 86 PSM subjects with prediabetes (pre-DM), and 86 PSM subjects with prediabetes treated with metformin (pre-DM metformin) were analyzed. During coronary angiography, NOCS was categorized by luminal stenosis <40% and fractional flow reserve >0.80. In addition, we assessed the endothelial function, measuring coronary artery diameter of left anterior descending coronary (LAD) at baseline and after the infusion of acetylcholine, by means of an intracoronary Doppler guide wire. MACE, as cardiac death, myocardial infarction, and heart failure, was evaluated at 24 months of follow-up.

RESULTS

At baseline, NG patients had a lower percentage of LAD endothelial dysfunction compared with pre-DM patients ($P < 0.05$). The pre-DM patients had a higher percentage of endothelial LAD dysfunction as compared with the pre-DM metformin patients ($P < 0.05$). At the 24th month of follow-up, MACE was higher in pre-DM versus NG ($P < 0.05$). In pre-DM metformin patients, MACE was lower compared with pre-DM patients ($P < 0.05$).

CONCLUSIONS

Metformin therapy may reduce the high risk of cardiovascular events in pre-DM patients by reducing coronary endothelial dysfunction.

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Received 13 November 2018 and accepted 22 January 2019

Clinical trial reg. no. NCT03553030, clinicaltrials.gov

This article is featured in a podcast available at http://www.diabetesjournals.org/content/diabetes-core-update-podcasts.

Prediabetes is an intermediate metabolic state between normoglycemia and diabetes (1). Prediabetes includes patients with impaired glucose tolerance and impaired fasting glucose and hemoglobin A_{1c} (HbA_{1c}) values between 5.7% and 6.4% (39–46 mmol/mol) (1). Worldwide, more than 400 million people have prediabetes, and projections indicate that by 2030 more than 470 million people will have prediabetes (2). Moreover, in a recent survey based on HbA_{1c} results, 33.6% of outpatients (out of 1.16 million outpatient visits analyzed) had prediabetes (2). Intriguingly, <1% of those patients whose HbA_{1c} tests showed prediabetes were recognized and diagnosed as such by clinicians (1,2). To date, with a growing trend, prediabetes affects >38% of people in the adult population, and it is associated with an increased risk of developing diabetes (3). Although some prospective studies have shown that prediabetes is associated with an increased risk of cardiovascular disease (4,5), other studies have not found a similar association (6–8). However, several previous meta-analyses have led to conflicting conclusions (3,9,10), due to differences in end point assessments and study inclusion criteria. In this context, a very recent study showed that prediabetes was not associated with an increased risk of subclinical coronary atherosclerosis (stenosis severity ≥50%) evaluated transversally by coronary computed tomographic angiography (11). Moreover, Liu et al. (12) showed that among patients with stable, new-onset coronary heart disease (stenosis severity ≥50%), the increased cardiovascular risk in prediabetes is largely affected by the coexistence of hypertension rather than prediabetes per se. Therefore, these studies did not provide any evidence about the role of prediabetes on cardiovascular outcomes in the early coronary atherosclerotic disease progression, such as those found in nonobstructive coronary stenosis (NOCS) (stenosis severity <50%), or assess the specific mechanisms transducing prediabetes environmental stimuli in coronary atherosclerotic disease progression. In this context, the common link between prediabetes and coronary heart disease may be represented by hyperglycemia and insulin resistance, both leading to the early insurgence of coronary artery dysfunction (13,14). Therefore, these pathological mechanisms may cause coronary artery dysfunction also in the absence of obstructive coronary stenosis. Recently, Sara et al. (15) showed that patients with stable angina, stable coronary artery disease (CAD), and NOCS have endothelial dysfunction, which is consequently linked to an increased rate of worse prognosis and cardiac mortality. However, a great disagreement exists in literature about the correlation between prediabetes, endothelial dysfunction, and clinical outcomes in stable CAD-NOCS. Thus, this study was designed to identify differences in endothelial coronary function, as well as major adverse cardiac events (MACE) at 24 months of follow-up, between patients with prediabetes and normoglycemic (NG) patients with stable angina and NOCS. In addition, American Diabetes Association (ADA) guidelines suggest that prediabetes be treated with hypoglycemic drugs such as metformin to control glucose homeostasis and to reduce the risk of diabetes development and the linked worse prognosis (16). Intriguingly, less is known about the effect of metformin to reduce the coronary endothelial dysfunction and the consequent improved clinical prognosis in patients with prediabetes with stable CAD-NOCS. Moreover, here we evaluated the effect of metformin therapy on coronary endothelial function and MACE in patients with prediabetes with stable angina and NOCS.

RESEARCH DESIGN AND METHODS

This is a multicenter prospective study conducted at the Department of Cardiology, Antonio Cardarelli Hospital, at the Department of Cardiovascular Diseases, John Paul II Research and Care Foundation (Campobasso, Italy), and at the Department of Internal Medicine and Metabolic Diseases, University of Campania "Luigi Vanvitelli." From January 2009 to January 2016, we screened patients having stable angina pectoris and stable angina with a positive stress test for myocardial ischemia; no change in the frequency, duration, or intensity of clinical symptoms within 4 weeks; and referred for elective coronary artery angiography. However, these patients received a coronary angiography, and 908 patients with evidence of coronary NOCS (<40%) and no physiologically significant fractional flow reserve (>0.80) were prospectively included in a database. Patients with no coronary disease detected by coronary angiography, presence of obstructive stenosis, left ventricular ejection fraction <50%, previous myocardial infarction, previous percutaneous coronary intervention and/or coronary bypass grafting, Takotsubo cardiomyopathy, myocarditis, impaired renal function, or stroke were instead excluded. Prediabetes was categorized according to the criteria of the ADA: fasting plasma glucose of ≥5.6 mmol/L but <7.0 mmol/L (100–125 mg/dL [impaired fasting glucose]), a 2-h glucose of ≥7.8 mmol/L but <11.1 mmol/L during a 75-g oral glucose tolerance test (140–199 mg/dL [impaired glucose tolerance]), or a plasma HbA_{1c} of ≥5.7% but <6.5% (16). Furthermore, patients with prediabetes answered a specific questionnaire about metformin treatment before the beginning of the study, the dates of beginning and end of treatment, and the duration of use. Information from the medicine inventory during the study and this specific questionnaire were used to classify the subjects. The patients with prediabetes who never used metformin were classified as "never metformin users." The patients with prediabetes who had already used metformin were classified as "current metformin users," and they had been treated with metformin for at least 6 months. Patients treated with metformin for <6 months were instead excluded from the study. Information on the duration of treatment was available for all current users. In all patients, we evaluated the endothelial coronary vascular function at baseline and after infusion of acetylcholine. The analyses of all angiographic data were performed by the interventional cardiologists (C.Sac., C.M., and F.M.), blinded to patient categorization, who reviewed selected cases. At the Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences (University of Campania "Luigi Vanvitelli"), we performed for all patients (as outpatients) a quarterly clinical evaluation, routine analyses, plasma glucose, and HbA_{1c} level measurements and cardiovascular evaluation for 24 months after the coronarography. The study end points were the assessment of oxidative stress, inflammatory

tone, and MACE at 24 months of follow-up. The study was conducted in accordance with the Declaration of Helsinki. The ethics committees of all participating institutions approved the protocol. All patients were informed about the study nature and gave their written informed and signed consent to participate in the study.

Coronary Angiography and Endothelial Function Assessment

Experienced physicians (C.Sac., C.M., and F.M.) performed routine diagnostic coronary angiography (Discovery IGS 740; General Electric) by using standard clinical protocols (15,17,18). Coronary angiography was performed to discriminate and select patients with NOCS, as a stenosis $<$40% of vessel lumen, and with a fractionated flow reserve $>$0.80 (15,17,18). After NOCS diagnosis, we evaluated the endothelial coronary vascular function at baseline and after each infusion of acetylcholine (15). In brief, by using an intracoronary Doppler guide wire advanced within the coronary infusion catheter and positioned in the midleft anterior descending coronary artery, we evaluated changes in the coronary blood flow (CBF) through the measurement of coronary artery diameter at baseline and after the infusion of acetylcholine (15). This protocol was performed by an independent investigator blinded to Doppler velocity data and using a previously described computer-based image analysis system (15). The infusion protocol of acetylcholine was terminated when the highest molar concentration of acetylcholine (1,024 mol/L) was reached (15). Endothelial-dependent CBF was then calculated by the following formula: CBF $=$ $0.25 \times \pi \times$ (average peak velocity) $\times$ (coronary artery diameter)$^2 \times 0.5$ (15). The interobserver and intraobserver reproducibility of the CBF calculation was $\sim$5%. The maximal percent increase in CBF in response to acetylcholine compared with the CBF at baseline was then calculated, and all measurements were performed in the segment 5 mm distal to the tip of the Doppler guidewire. Moreover, after each acetylcholine infusion, the coronary artery diameter was measured in the same segment of the vessel. The maximal effect of acetylcholine was expressed as percent change in coronary artery diameter using quantitative coronary angiography (Medis Corporation, Leiden, the Netherlands) (representing epicardial endothelial function) and percent change in the CBF (representing microvascular endothelial function) relative to baseline (15).

Biochemical Analyses

Venous blood samples obtained from all participants in the study at baseline and during follow-up phases were centrifuged at 3,000 rotations/min, and serum/plasma samples were collected and stored at $-$80°C until assayed. Serum levels of hs-CRP, interleukin 1 (IL1) and 6 (IL6), and tumor necrosis factor (TNF)α were measured as inflammatory biomarkers. In addition, we measured the number of white blood cells (WBCs), granulocytes, platelets, and blood values of nitrotyrosine as markers of oxidative stress on admission, before coronary angiography, and at follow-up (17,18).

Statistical Analysis

SPSS Statistics, version 23.0 (IBM), was used for all statistical analyses. Categorical variables were presented as number and percentage and continuous variables as mean $\pm$ SD. The study sample size of NG subjects and subjects with prediabetes was calculated using a power of 80% and CI of 95%. Propensity score matching (PSM) was developed to compare NG subjects, subjects with prediabetes (pre-DM), and subjects with prediabetes treated with metformin (pre-DM metformin) from the predicted probabilities of MACE by a multivariable logistic regression model. NG subjects were matched to pre-DM and to pre-DM metformin subjects based on PSM. In all matched patients, the balancing property was satisfied, and PSM was developed from the predicted probabilities of a multivariable logistic regression model predicting MACE according to age, sex,

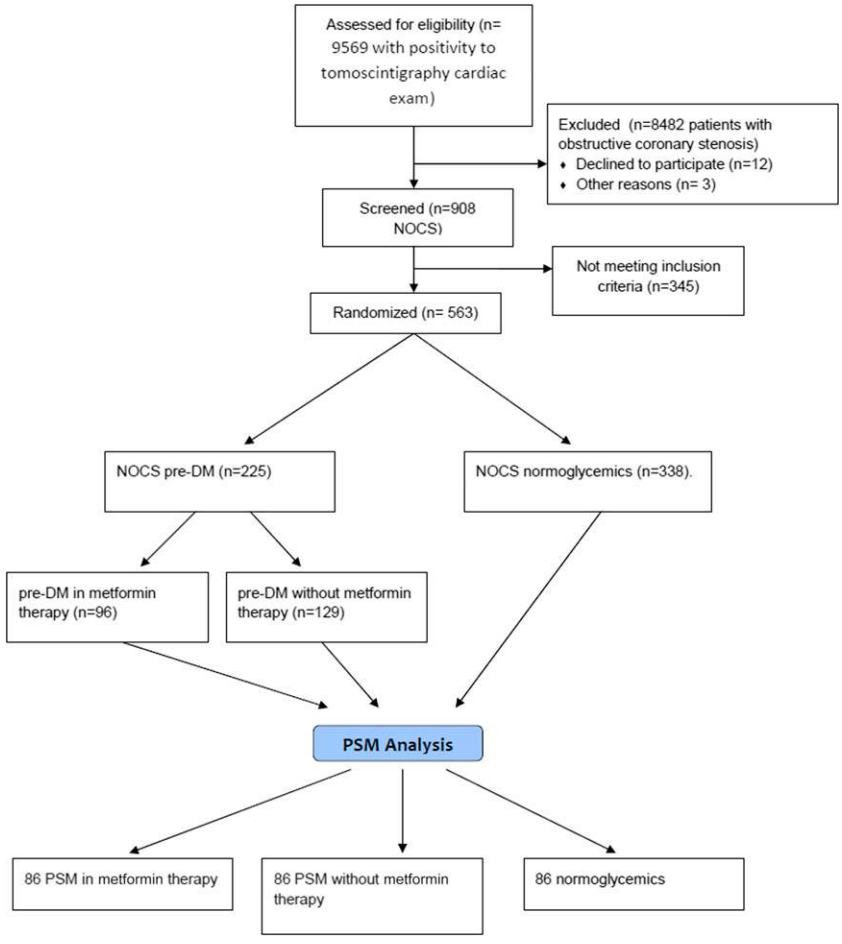

Figure 1—Study diagram: phases of enrollment, allocation, follow-up, and analysis.

hypertension, dyslipidemia, smoking history, family history, baseline therapies, metabolic characteristics, and coronary lesions. Overall survival and event-free survival were assessed by Kaplan-Meier survival curves and compared by the log-rank test. Univariable Cox models were then used to compare event risks. The resulting hazard ratios (HRs) and 95% CIs were reported. Two-tailed *P* values <0.05 were considered statistically significant.

RESULTS

A total of 9,569 patients were submitted to elective coronary angiographic study for stable angina and positive stress test for myocardial ischemia between January 2009 and June 2016. Of these, 1,087

Table 1—Clinical characteristics of study population at baseline

Clinical variables	86 PSM NG patients	86 PSM pre-DM patients	86 PSM pre-DM metformin patients	*P*
General characteristics				
Age (years)	65.4 ± 5.5	65.5 ± 5.9	64.9 ± 6.1	n.s.
BMI (kg/m^2)	29.5 ± 1.85	29.7 ± 1.9	29.5 ± 1.88	n.s.
Systolic blood pressure (mmHg)	127.5 ± 8.4	126.2 ± 10.7	125.2 ± 10.3	n.s.
Diastolic blood pressure (mmHg)	79.5 ± 8.8	79.1 ± 6.5	78.3 ± 5.9	n.s.
Heart rate	85.1 ± 7.4	85.5 ± 8.8	86.1 ± 8.1	n.s.
Biochemical measurements				
Glucose (mmol/L)	4.98 ± 0.47	6.32 ± 0.36	5.57 ± 0.41	<0.05*†‡
HbA_{1c} (%)	5.1 ± 0.82	6.1 ± 0.54	5.41 ± 0.66	<0.05*†‡
HbA_{1c} (mmol/mol)	32.2 ± 5.01	43.3 ± 3.79	35.4 ± 4.82	<0.05*†‡
Cholesterol (mmol/L)	5.32 ± 0.5	5.31 ± 0.5	5.30 ± 0.6	n.s.
HDL (mmol/L)	0.99 ± 0.08	0.96 ± 0.09	0.95 ± 0.07	n.s.
LDL (mmol/L)	3.38 ± 0.51	3.42 ± 0.50	3.39 ± 0.48	n.s.
Triglycerides (mmol/L)	2.06 ± 0.22	2.05 ± 0.25	2.05 ± 0.21	n.s.
Creatinine (μmol/L)	87.1 ± 13.2	87.1 ± 14.1	88.2 ± 14.3	n.s.
Cardiovascular risk factors, *n* (%)				
Hypertension	59 (68.6)	59 (68.6)	59 (68.6)	n.s.
Dyslipidemia	39 (45.3)	39 (45.3)	39 (45.3)	n.s.
Smokers	62 (72)	62 (72)	62 (72)	n.s.
Medication, *n* (%)				
β-Blockers	32 (37.8)	32 (37.2)	32 (37.2)	n.s.
ACE inhibitors	24 (27.9)	24 (27.9)	24 (27.9)	n.s.
Angiotensin receptor blockers	22 (25.6)	22 (25.6)	22 (25.6)	n.s.
Calcium blockers	19 (22.1)	19 (22.1)	19 (22.1)	n.s.
Statins	35 (40.7)	35 (40.7)	35 (40.7)	n.s.
Diuretics	9 (10.5)	9 (10.5)	9 (10.5)	n.s.
Aspirin	47 (54.6)	47 (54.6)	47 (54.6)	n.s.
Metformin			86 (100)	
Inflammatory markers				
WBCs (10^9/L)	6.65 ± 0.71	7.72 ± 0.77	7.12 ± 0.68	<0.05*†‡
Granulocytes (10^9/L)	4.11 ± 0.66	4.74 ± 0.62	4.59 ± 0.69	<0.05*†‡
Monocytes (10^9/L)	0.39 ± 0.04	0.43 ± 0.06	0.42 ± 0.07	<0.05*†‡
Platelets (10^9/L)	272 ± 25	277 ± 25	279 ± 25	n.s.
Fibrinogen (mg/dL)	331 ± 27	354 ± 42	345 ± 30	n.s.
CRP (mg/L)	2.13 ± 0.52	3.69 ± 0.72	3.21 ± 0.63	<0.05*†‡
IL1 (pg/dL)	295.9 ± 52.1	365.8 ± 87.8	328.5 ± 67.3	<0.05*†‡
IL6 (pg/dL)	188.4 ± 19.7	234.3 ± 44.2	223.5 ± 24.1	<0.05*†‡
TNFα (mg/dL)	3.56 ± 1.12	5.55 ± 0.78	4.76 ± 0.93	<0.05*†‡
Nitrotyrosine (mg/dL)	0.41 ± 0.1	0.53 ± 0.1	0.49 ± 0.2	<0.05*†‡
Epicardial endothelial vessel characteristics				
Lumen area, mm^2	15.92 ± 3.09	11.16 ± 2.07	11.76 ± 3.07	<0.05*‡
Reference diameter	2.81 ± 0.48	2.58 ± 0.47	2.61 ± 0.47	<0.05*‡
Flow (estimated in mL/s)	1.24 ± 0.59	1.03 ± 0.41	1.18 ± 0.47	<0.05*†‡
Epicardial endothelial dysfunction, *n* (%)	25 (29.1)	63 (73.2)	41 (47.7)	<0.05*†‡
Plaque characteristics				
Plaque area, mm^2	3.31 ± 2.12	3.48 ± 2.51	3.38 ± 2.31	n.s.
Plaque burden, %	24.42 ± 12.03	24.22 ± 10.86	24.29 ± 11.15	n.s.
Minimum lumen area, mm^2	9.49 ± 3.61	9.52 ± 3.87	9.50 ± 3.67	n.s.
Plaque thickness, mm	0.34 ± 0.21	0.35 ± 0.22	0.34 ± 0.63	n.s.
Maximum plaque burden per artery, %	34.52 ± 13.87	35.31 ± 14.93	35.11 ± 14.25	n.s.
Minimum lumen area per artery, mm^2	6.94 ± 3.27	7.12 ± 4.15	7.07 ± 3.86	n.s.
Maximum plaque thickness per artery, mm	0.57 ± 0.32	0.59 ± 0.36	0.58 ± 0.63	n.s.

Data are means ± SD unless otherwise indicated. **P* value <0.05, NG vs. pre-DM patients; †*P* value <0.05, pre-DM vs. pre-DM metformin patients; ‡*P* value <0.05, NG vs. pre-DM metformin patients.

presented NOCS (699 without diabetes and 388 with diabetes). Among these patients, 345 did not meet inclusion criteria. Therefore, the final study population comprised 563 patients (225 with prediabetes and 338 NG). Among patients with prediabetes, 96 were current metformin users and 129 were never metformin users. After PSM for metabolic and cardiovascular risk factors, 86 current metformin users were matched to 86 never metformin users and 86 NG patients (Fig. 1). Among the current metformin users, the mean ± SD duration of incretin treatment was 37 ± 6 months. Study population characteristics are reported in Table 1.

At baseline, prediabetes versus NG patients had higher values of glucose and HbA_{1c} ($P < 0.05$) (Table 1). NG versus pre-DM and NG versus pre-DM metformin had lower values of WBCs ($P < 0.05$), granulocytes ($P < 0.05$), monocytes ($P < 0.05$), C-reactive protein (CRP) ($P < 0.05$), IL1 ($P < 0.05$), IL6 ($P < 0.05$), TNFα ($P < 0.05$), and nitrotyrosine ($P < 0.05$) (Table 1).

With regard to the epicardial endothelial vessel characteristics, pre-DM and pre-DM metformin versus NG patients had smaller lumen area ($P < 0.05$) and reference diameter ($P < 0.05$), lower flow ($P < 0.05$), and a higher percentage of epicardial endothelial dysfunction ($P < 0.05$) (Table 1). In addition, pre-DM versus pre-DM metformin showed a lower epicardial coronary flow ($P < 0.05$) and a higher percentage of epicardial endothelial dysfunction ($P < 0.05$) (Table 1).

Changes in CBF

The acetylcholine-induced percentage changes in CBF in the three groups are reported in Fig. 2. There were significant differences between pre-DM patients (−56.4 ± 6.1%) compared with NG and pre-DM metformin (172.4 ± 18.3% and 19.2 ± 2.5%, respectively, $P < 0.05$) (Fig. 2). There were also significant differences between NG and pre-DM metformin. The acetylcholine-induced percent changes in coronary artery diameter also revealed significant differences between the three groups (5.5 ± 2.6%, −21.5 ± 2.9%, and −49.4 ± 3.4% in NG, pre-DM metformin, and pre-DM, respectively, $P < 0.05$) (Fig. 2). As reported in the text, we performed noninvasive functional studies in all the patients studied before performing the coronary angiography. However, we did not find significant differences in the prevalence of positive noninvasive functional studies between the study groups. The coronary flow reserve to adenosine was significantly lower in pre-DM (2.1 ± 0.1) compared with NG and pre-DM metformin. To date, the three study groups presented no significant differences in systemic hemodynamic parameters (mean arterial pressure and heart rate).

At the sixth month of follow-up, we reported a statistically significant reduction of glucose blood levels ($P < 0.05$) and of HbA_{1c} ($P < 0.05$) in pre-DM metformin versus pre-DM (Table 2). Consequently, there was an overexpression of inflammatory markers and MACE in pre-DM versus pre-DM metformin, as well as in comparison of pre-DM with NG patients. However, pre-DM versus pre-DM metformin, and pre-DM versus NG, patients had higher values of WBCs ($P < 0.05$), granulocytes ($P < 0.05$), monocytes ($P < 0.05$), CRP ($P < 0.05$), IL1 ($P < 0.05$), IL6 ($P < 0.05$), and TNFα ($P < 0.05$) (Table 2). Moreover, nitrotyrosine values were also higher in pre-DM versus pre-DM metformin ($P < 0.05$), in pre-DM versus NG ($P < 0.05$), and in pre-DM metformin versus NG ($P < 0.05$) patients. Also, the number and percentage of MACE were higher in pre-DM versus pre-DM metformin ($P < 0.05$), in pre-DM versus NG ($P < 0.05$), and in pre-DM metformin versus NG ($P < 0.05$) patients (Tables 2 and 4). Two pre-DM metformin patients (2.3%) vs. 4 pre-DM patients (4.6%) without hypoglycemic drug therapy became diabetic ($P < 0.05$) (Table 2).

At the 12th month of follow up, we reported a statistical significant reduction of glucose blood levels (P value <0.05) and of HbA_{1c} (P value <0.05) in pre-DM metformin versus pre-DM (Table 2). To date, we reported a statistically significant overexpression of inflammatory markers and MACE in pre-DM versus pre-DM metformin ($P < 0.05$) and pre-DM versus NG ($P < 0.05$) patients. However, pre-DM

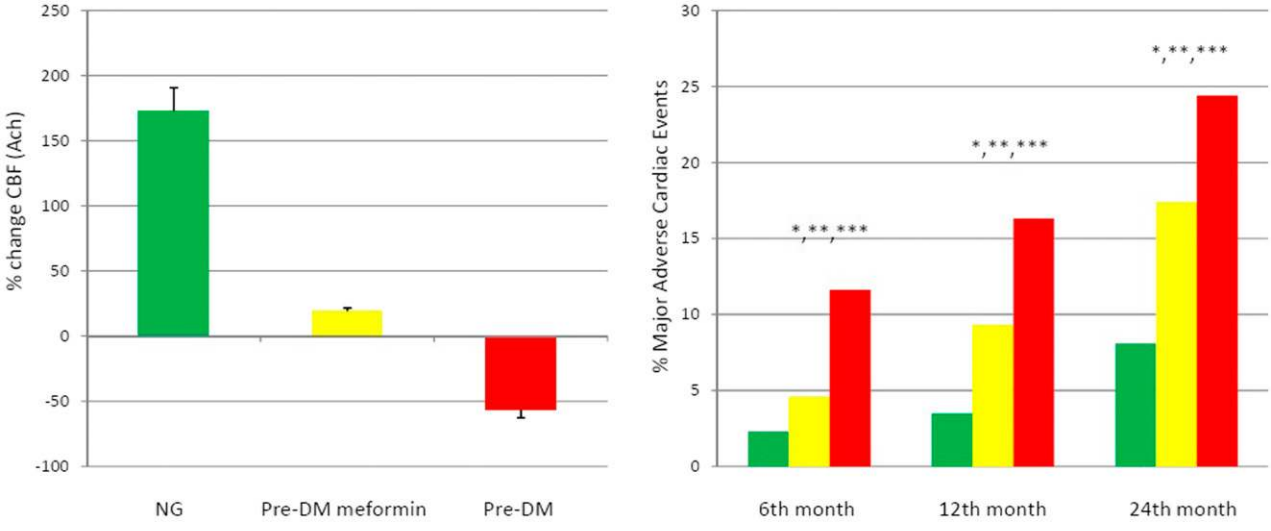

Figure 2—*A*: Mean percent change in CBF in response to acetylcholine (Ach) among three groups. *B*: MACE at 6th, 12th, and 24th months of follow-up. *$P < 0.05$, NG vs. pre-DM patients; **$P < 0.05$, pre-DM vs. pre-DM metformin patients; ***$P < 0.05$ NG vs. pre-DM metformin patients. Green, NG; yellow, pre-DM metformin; red, pre-DM.

Table 2—Inflammatory markers and MACE of study population at follow-up (6th, 12th, and 24th month)

	86 PSM NG patients	86 PSM pre-DM patients	86 PSM pre-DM metformin patients	*P*
6th month				
Biochemical measurements				
Glucose (mmol/L)	4.94 ± 0.41	6.31 ± 0.32	5.11 ± 0.46	<0.05*†
HbA_{1c} (%)	5.1 ± 0.68	6.1 ± 0.54	5.3 ± 0.51	<0.05*†
HbA_{1c} (mmol/mol)	32.4 ± 4.91	43.2 ± 3.63	34.5 ± 4.25	<0.05*†
Cholesterol (mmol/L)	5.12 ± 0.5	5.11 ± 0.5	5.10 ± 0.6	n.s.
HDL (mmol/L)	0.89 ± 0.07	0.86 ± 0.09	0.85 ± 0.07	n.s.
LDL (mmol/L)	3.23 ± 0.47	3.21 ± 0.48	3.22 ± 0.43	n.s.
Triglycerides (mmol/L)	1.96 ± 0.22	1.98 ± 0.21	1.99 ± 0.23	n.s.
Creatinine (μmol/L)	88.2 ± 13.3	88.3 ± 15.3	93.2 ± 14.3	n.s.
Diabetes		2 (2.3)	4 (4.6)	<0.05*†‡
Inflammatory markers				
WBCs (10^9/L)	6.41 ± 0.58	7.35 ± 0.66	6.49 ± 0.63	<0.05*†
Granulocytes (10^9/L)	3.89 ± 0.36	4.19 ± 0.51	3.92 ± 0.61	<0.05*†
Monocytes (10^9/L)	0.33 ± 0.09	0.38 ± 0.03	0.34 ± 0.07	<0.05*†
Platelets (10^9/L)	232 ± 22	223 ± 19	229 ± 26	n.s.
Fibrinogen (mg/dL)	321 ± 27	334 ± 42	322 ± 31	<0.05*†
CRP (mg/L)	1.92 ± 0.73	2.36 ± 0.77	2.05 ± 0.63	<0.05*†
IL1 (pg/dL)	212.9 ± 41.3	278.8 ± 69.6	218.5 ± 63.6	<0.05*†
IL6 (pg/dL)	138.4 ± 17.2	194.3 ± 41.7	149.5 ± 19.3	<0.05*†
TNFα (mg/dL)	2.25 ± 0.92	4.32 ± 0.62	2.76 ± 0.93	<0.05*†
Nitrotyrosine (mg/dL)	0.26 ± 0.08	0.38 ± 0.05	0.34 ± 0.11	<0.05*†‡
MACE	2 (2.3)	10 (11.6)	4 (4.6)	<0.05*†‡
12th month				
Biochemical measurements				
Glucose (mmol/L)	4.92 ± 0.37	6.26 ± 0.35	5.10 ± 0.37	<0.05*†
HbA_{1c} (%)	5.1 ± 0.58	6.1 ± 0.46	5.2 ± 0.89	<0.05*†
HbA_{1c} (mmol/mol)	32.4 ± 4.85	43.2 ± 3.58	33.8 ± 4.91	<0.05*†
Cholesterol (mmol/L)	5.12 ± 0.5	5.11 ± 0.5	5.10 ± 0.6	n.s.
HDL (mmol/L)	0.89 ± 0.07	0.86 ± 0.09	0.85 ± 0.07	n.s.
LDL (mmol/L)	3.23 ± 0.47	3.21 ± 0.48	3.22 ± 0.43	n.s.
Triglycerides (mmol/L)	1.96 ± 0.22	1.98 ± 0.21	1.99 ± 0.23	n.s.
Creatinine (μmol/L)	88.7 ± 13.5	88.6 ± 15.5	96.2 ± 13.8	n.s.
Diabetes	1 (1.1)	3 (3.5)	8 (9.3)	<0.05*†‡
Inflammatory markers				
WBCs (10^9/L)	6.22 ± 0.52	7.27 ± 0.61	6.29 ± 0.63	<0.05*†
Granulocytes (10^9/L)	3.72 ± 0.31	4.08 ± 0.47	3.88 ± 0.49	<0.05*†
Monocytes (10^9/L)	0.31 ± 0.05	0.33 ± 0.02	0.32 ± 0.05	n.s.
Platelets (10^9/L)	225 ± 24	221 ± 22	229 ± 26	n.s.
Fibrinogen (mg/dL)	318 ± 25	323 ± 39	321 ± 28	n.s.
CRP (mg/L)	1.89 ± 0.71	2.32 ± 0.71	1.93 ± 0.59	<0.05*†
IL1 (pg/dL)	208.7 ± 39.5	271.5 ± 66.5	227.6 ± 43.8	<0.05*†
IL6 (pg/dL)	135.6 ± 16.8	189.1 ± 39.5	144.4 ± 17.6	<0.05*†
TNFα (mg/dL)	2.19 ± 0.88	4.21 ± 0.58	2.36 ± 0.74	<0.05*†
Nitrotyrosine (mg/dL)	0.23 ± 0.07	0.32 ± 0.04	0.26 ± 0.11	<0.05*‡
MACE	3 (3.5)	14 (16.3)	8 (9.3)	<0.05*†‡
24th month				
Biochemical measurements				
Glucose (mmol/L)	4.91 ± 0.35	6.22 ± 0.38	5.09 ± 0.39	<0.05*†
HbA_{1c} (%)	5.1 ± 0.46	6.1 ± 0.42	5.2 ± 0.59	<0.05*†
HbA_{1c} (mmol/mol)	32.4 ± 4.79	43.2 ± 3.56	33.8 ± 4.73	<0.05*†
Cholesterol (mmol/L)	5.10 ± 0.4	5.10 ± 0.5	5.09 ± 0.6	n.s.
HDL (mmol/L)	0.87 ± 0.06	0.81 ± 0.06	0.81 ± 0.09	n.s.
LDL (mmol/L)	3.18 ± 0.46	3.17 ± 0.41	3.18 ± 0.39	n.s.
Triglycerides (mmol/L)	1.93 ± 0.27	1.97 ± 0.19	1.97 ± 0.25	n.s.
Creatinine (μmol/L)	88.7 ± 13.5	88.6 ± 15.5	96.2 ± 13.8	n.s.
Diabetes	2 (2.3)	5 (5.8)	15 (17.4)	<0.05*†‡
Inflammatory markers				
WBCs (10^9/L)	6.18 ± 0.48	7.24 ± 0.59	6.22 ± 0.55	<0.05*†
Granulocytes (10^9/L)	3.26 ± 0.26	3.97 ± 0.42	3.46 ± 0.43	<0.05*†
Monocytes (10^9/L)	0.24 ± 0.03	0.31 ± 0.18	0.29 ± 0.09	n.s.
Platelets (10^9/L)	222 ± 26	225 ± 27	221 ± 24	n.s.
Fibrinogen (mg/dL)	309 ± 22	318 ± 37	316 ± 21	n.s.

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Table 2—Continued

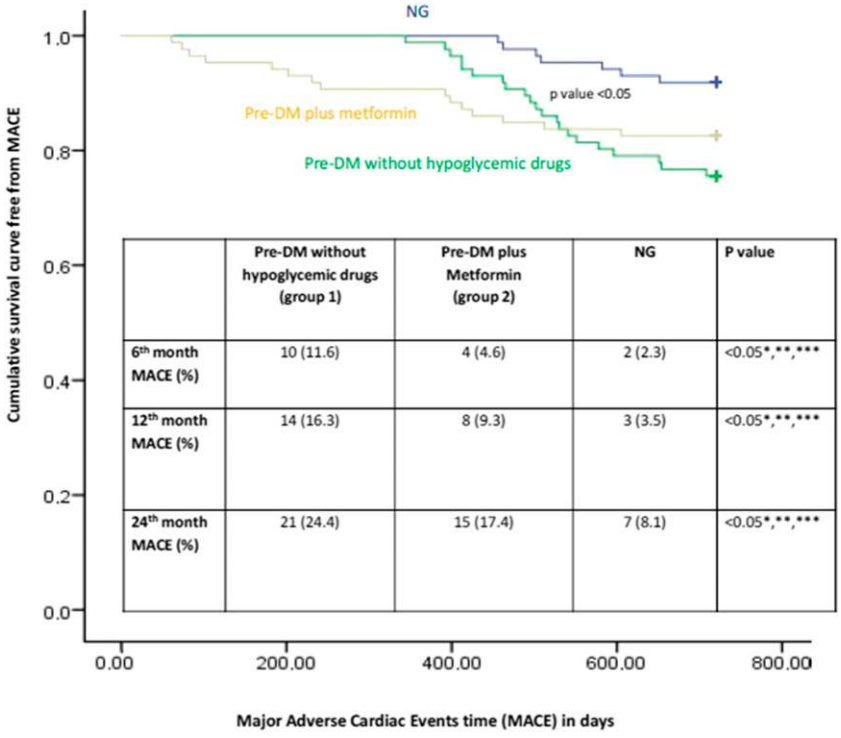
	86 PSM NG patients	86 PSM pre-DM patients	86 PSM pre-DM metformin patients	*P*
CRP (mg/L)	1.76 ± 0.56	2.27 ± 0.58	1.81 ± 0.55	<0.05*†
IL1 (pg/dL)	202.4 ± 33.9	268.3 ± 61.6	211.3 ± 40.6	<0.05*†
IL6 (pg/dL)	132.7 ± 15.6	186.2 ± 34.7	134.4 ± 16.9	<0.05*†
TNFα (mg/dL)	2.11 ± 0.63	4.12 ± 0.63	2.16 ± 0.67	<0.05*†
Nitrotyrosine (mg/dL)	0.21 ± 0.04	0.31 ± 0.01	0.22 ± 0.15	<0.05*†
MACE	7 (8.1)	21 (24.4)	15 (17.4)	<0.05*†‡

Data are means ± SD or *n* (%). **P* value <0.05, NG vs. pre-DM patients; †*P* value <0.05, pre-DM vs. pre-DM metformin patients; ‡*P* value <0.05, NG vs. pre-DM metformin patients.

versus pre-DM metformin and pre-DM versus NG patients had higher values of WBCs ($P < 0.05$), granulocytes ($P < 0.05$), CRP ($P < 0.05$), IL1 ($P < 0.05$), IL6 ($P < 0.05$), TNFα ($P < 0.05$), and nitrotyrosine ($P < 0.05$) (Table 2). Also, the number and percentage of MACE were higher in pre-DM versus pre-DM metformin ($P < 0.05$), in pre-DM versus NG ($P < 0.05$), and in pre-DM metformin versus NG ($P < 0.05$) patients (Table 2 and Fig. 3). One (1.1%) NG versus three (3.5%) pre-DM metformin versus eight (9.3%) pre-DM patients without hypoglycemic drug therapy became diabetic (<0.05) (Table 2).

At 24 months of follow-up, pre-DM metformin versus pre-DM patients still maintained a statistically significant reduction of glucose blood levels ($P < 0.05$) and of HbA_{1c} ($P < 0.05$) (Table 1). However, we reported a statistically significant overexpression of inflammatory markers and of MACE in pre-DM versus pre-DM metformin and in pre-DM versus NG patients. However, pre-DM versus pre-DM metformin, and pre-DM versus NG, patients had higher values of WBCs ($P < 0.05$), granulocytes ($P < 0.05$), CRP ($P < 0.05$), IL1 ($P < 0.05$), IL6 ($P < 0.05$), TNFα ($P < 0.05$), and nitrotyrosine ($P < 0.05$) (Table 2). Also, the number and percentage of MACE were higher in pre-DM versus pre-DM metformin ($P < 0.05$), in pre-DM versus NG ($P < 0.05$), and in pre-DM metformin versus NG (15 [17.4%] vs. 7 [8.1%], $P < 0.05$) patients (Table 2 and Fig. 2). Two (2.3%) NG vs. 5 (5.8%) pre-DM metformin vs. 15 (17.4%) pre-DM patients without hypoglycemic drug therapy became diabetic ($P < 0.05$) (Table 2).

At the multivariate Cox regression analysis, MACE at 24 months of follow-up were predicted by CRP values (HR 1.543 [CI 95% 1.151–2.070], $P < 0.05$), IL1 values (1.195 [1.086–1.999], $P < 0.05$), IL16 values (1.140 [1.007–1.210], $P < 0.05$), WBCs (3.983 [2.322–6.833], $P < 0.05$), pre-DM (3.517 [1.858–6.658], $P < 0.05$), metformin therapy (0.619 [0.377–0.905], $P < 0.05$), and nitrotyrosine values (3.380 [2.837–4.761], $P = 0.05$) (Table 3 and Fig. 3). Intriguingly, higher glucose blood levels are associated with lower coronary artery flow in comparison of pre-DM vs. NG ($P < 0.05$) and pre-DM vs. pre-DM metformin ($P < 0.05$) (Fig. 4).



	Pre-DM without hypoglycemic drugs (group 1)	Pre-DM plus Metformin (group 2)	NG	P value
6th month MACE (%)	10 (11.6)	4 (4.6)	2 (2.3)	<0.05*,**,***
12th month MACE (%)	14 (16.3)	8 (9.3)	3 (3.5)	<0.05*,**,***
24th month MACE (%)	21 (24.4)	15 (17.4)	7 (8.1)	<0.05*,**,***

Figure 3—Kaplan survival curve of cumulative freedom from comparison of pre-DM (red) with pre-DM metformin (blue) patients and NG patients (green) at 24 months of follow-up. Asterisks mark a statistically significant value. MACE percentage: **P* value <0.05 NG vs. pre-DM patients; ***P* value <0.05, pre-DM vs. pre-DM metformin patients; ****P* value <0.05, NG vs. pre-DM metformin patients.

CONCLUSIONS

The first relevant finding of this study was that pre-DM patients have a higher rate of coronary endothelial dysfunction compared with NG patients in the context of stable CAD-NOCS. Indeed, epicardial endothelial-dependent vasodilatation, induced by intracoronary infusion of acetylcholine, was significantly impaired in pre-DM compared with NG patients.

As background for this association, hyperglycemia and insulin resistance, typical of prediabetes status, may play a pivotal role in increasing both oxidative stress and inflammation in coronary milieu of patients with prediabetes. In this context, we observed that nitrotyrosine, a marker of oxidative stress, inflammatory cells, and cytokines was higher in patients with prediabetes compared with NG patients. Moreover, nitrotyrosine was also associated with higher coronary endothelial dysfunction. A previous study (19) has reported that

Table 3—Univariate Cox regression analysis for MACE at 24-month follow-up

Variables	Multivariate analysis	*P*	Univariate analysis	*P*
Age	0.989 (0.945–1.036)	0.650	1.016 (0.963–1.070)	0.565
BMI	1.179 (1.041–1.335)	0.009	0.980 (0.840–1.143)	0.795
CRP	2.225 (1.736–2.851)	0.001	1.543 (1.151–2.070)	0.004*
Glycemia	1.053 (1.030–1.075)	0.001	1.007 (0.973–1.041)	0.703
IL1	1.010 (0.998–1.003)	0.739	1.195 1.086–1.999)	0.001*
IL6	1.017 (1.012–1.021)	0.001	1.140 (1.007–1.210)	0.001*
Metformin therapy	1.228 (0.782–1.927)	0.373	0.619 (0.377–0.905)	0.05*
Nitrotyrosine	62.411 (7.891–488.022)	0.001	3.380 (2.837–4.761)	0.05*
Prediabetes	6.001 (3.906–9.228)	0.001	3.517 (1.858–6.658)	0.001*
TNFα	1.601 (1.296–1.975)	0.001	1.106 (0.841–1.456)	0.471
WBCs	3.085 (2.141–4.446)	0.001	3.983 (2.322–6.833)	0.001*

Data are HR (95% CI).

insulin resistance is associated with endothelial dysfunction and confers independent prognostic information in patients without diabetes with chest pain without myocardial perfusion defects. However, this study did not provide any evidence about coronary endothelial dysfunction, such as that found in patients with prediabetes, or assess the specific pathway transducing prediabetes coronary environmental stimuli in stable CAD-NOCS poor outcomes. Our data suggest that in pre-DM, the hyperglycemia and the insulin resistance might lead to endothelial dysfunction in the absence of severe coronary stenosis by alterations in vasomotor tone and by the overproduction of inflammatory molecules and reactive oxygen species, as previously evidenced (14). Consequently, all these inflammatory molecules may lead to a subclinical endothelial function in the context of NOCS in patients with prediabetes. All this might increase the risk for the initiation and progression of coronary atherosclerosis in patients with prediabetes in the absence of severe coronary stenosis. In this setting, the second major finding of the study was that the pre-DM, versus NG, subjects evidenced a higher rate of MACE at the 6th, 12th, and 24th months of follow up. Different studies may explain the complex association existing between coronary endothelial dysfunction and MACE. First, we have to image the coronary endothelium as a physical barrier between the flowing blood stream and the thrombogenic subendothelial matrix and as a dynamic tissue with vasodilatative and antiadhesion properties induced by nitric oxide and interleukins and expressing anticoagulant properties (15,20). However, this strengthens the hypothesis that coronary endothelial dysfunction from one side is associated with many cardiovascular risk factors and from the other side is also itself a key factor for both the initiation and progression of atherosclerosis (16,17). Conversely, it is well known that endothelial dysfunction might cause an increased rate of cardiac events also in the absence of obstructive CAD (18). In fact, in NOCS patients the endothelial dysfunction leads to cardiac events by myocardial ischemia and acceleration of coronary atherosclerosis, such as assessed by the reduced CBF response to the infusion of acetylcholine (18). In our study, we have investigated in pre-DM versus NG the inflammatory/oxidative axis as the main factor leading to endothelial dysfunction and MACE in stable CAD-NOCS patients. The higher rate of endothelial dysfunction in pre-DM, as unmasked by acetylcoline infusion during coronarography, may lead in stable CAD-NOCS to the acceleration of coronary atherosclerosis (16–18), which might be linked to a higher rate of MACE at 24 months of follow-up. In line with this observation, baseline IL6 values were predictive of MACE at 24 months of follow-up. Previously, authors showed that, in pre-DM, the baseline overexpression of IL6 and the endothelial molecular and cellular dysfunction caused an abnormal prothrombotic state and an advanced atherogenesis of the coronary vessels (14). In line with these study results, here we report the overexpression of WBCs and granulocytes cells in pre-DM versus NG. These cellular lines are active in the production and in the secretion of inflammatory and pro-oxidative molecules, therefore contributing to coronary vessel chronic inflammation (14,21–26). These cells in patients with prediabetes with stable CAD-NOCS might secrete inflammatory cytokines such as TNFα and IL6 (27), which then activate the NADPH oxidase, which is involved in nitrotyrosine synthesis (25–27). Nitrotyrosine is a marker of oxidative stress, as well as of endothelial dysfunction, and it is induced by altered glucose homeostasis (7) and enhanced by hyperactivity of

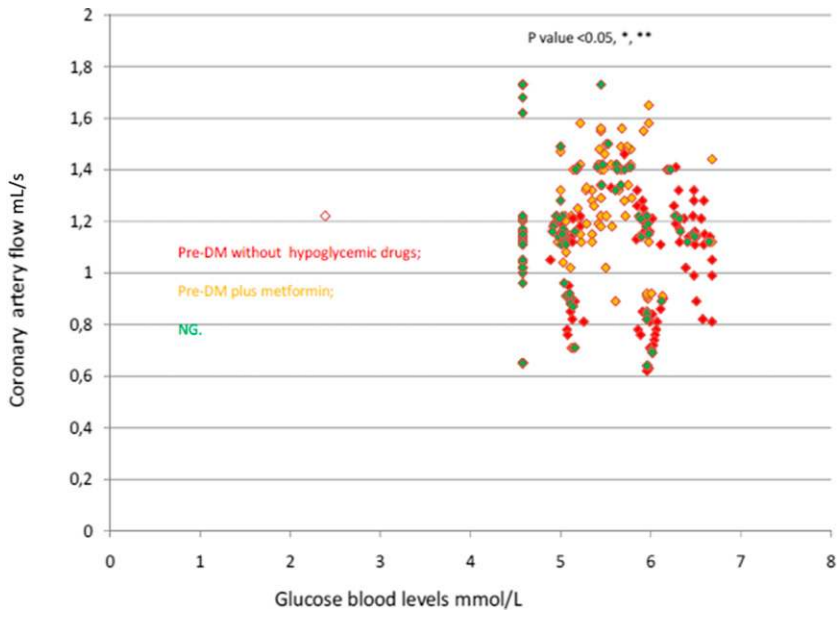


Figure 4—Dispersion graphic curve for endothelial blood flow in mL/s (*y*-axis), and glucose blood values in mmol/L (*x*-axis) at enrollment in pre-DM (red), pre-DM metformin (orange), and NG (green) patients.

Table 4—Most relevant study points and effects on endothelial function

Most relevant study points	Effects on endothelial function
Prediabetes	The patients with prediabetes by altered glucose and HbA_{1c} blood values show a higher rate of oxidative stress and inflammation that may lead to the endothelial dysfunction in NOCS.
Oxidative stress	A higher level of oxidative stress by increased nitrotyrosine (mg/dL) blood values might negatively condition the endothelial function in NOCS patients.
Inflammatory status	The inflammatory status by increased number of blood inflammatory cells (WBCs, granulocytes, and monocytes) and inflammatory proteins and cytokines (CRP, IL1, IL6, TNFα) might cause a higher rate of endothelial dysfunction in NOCS patients.
Metformin therapy	The metformin therapy may reduce inflammation/oxidative stress in patients with prediabetes, and this could likely ameliorate endothelial function, which will then reduce the MACE.

NADPH oxidase (26,27). Nitrotyrosine along with other proinflammatory molecules might regulate the atherosclerotic plaque instability and progression (14). However, inflammatory/oxidative stress and inflammatory cell overactivation cause a negative circle, which favors all these pathogenic processes, the endothelial dysfunction, and the worse prognosis in pre-DM versus NG patients. In this setting, it is relevant to show the endothelial dysfunction as a dynamic process in those with prediabetes, reversible at every phase by tailored treatments (6). (Table 4 reports on the correlation with endothelial function of co-/shared risk factors for cardiovascular disease.) Here, the major finding of our study is that metformin therapy may downregulate the inflammation/oxidative stress, hence reducing MACE rate at 24 months of follow-up in those with prediabetes with stable CAD-NOCS. This may represent a relevant study result because it should be evaluated in the context of new scenario and opportunity of treatments for patients with prediabetes with stable CAD-NOCS. However, this is not the first time that metformin has been proposed as a drug to reduce the inflammation and cell adhesion molecules in patients with impaired glucose homeostasis and stable CAD (28). Conversely, metformin effects in stable coronary atherosclerosis are well known and established (28). On the other hand, we report for the first time in the literature metformin's effects in pre-DM with stable CAD-NOCS. Therefore, treatment with metformin 850 mg twice a day for prediabetes with stable CAD-NOCS resulted in the reduction of MACE of ~40%. Thus, metformin therapy in prediabetes ameliorates not only the glucose blood levels and HbA_{1c} values but also the expression of all inflammatory/oxidative molecules and, consequently, the rate of MACE at 24 months of follow-up. Although ADA guidelines suggest that patients with prediabetes be treated with metformin to reduce the risk of developing diabetes (16), to date metformin use is <1% among adults with prediabetes and only slightly more common among those with additional risk factors for diabetes (29). In this context, our data may help to reduce the important gap in the prevention and treatment of coronary disease induced by dysglycemia and insulin resistance. In the future, studies will be conducted on a greater number of patients with prediabetes, and with a longer follow-up, to best assess all these molecular and clinical alterations.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.Sar. contributed to design of the study and research project and data collection and analysis and wrote the manuscript. P.P. contributed to data collection. C.Sac. and F.M. performed cardiac scintigraphy imaging. C.Sac., C.M., F.M., and M.P. performed coronarography. N.D. and M.L.B. performed biochemical analysis. M.R.R., M.B., F.C.S., P.C., G.P., and R.M. contributed to manuscript revision and editing. C.Sar. and R.M. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for developing diabetes. Lancet 2012;379:2279–2290
2. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 2016;387:1513–1530
3. Knowler WC, Barrett-Connor E, Fowler SE, et al.; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403
4. Matsuzawa Y, Li J, Aoki T, et al. Predictive value of endothelial function by noninvasive peripheral arterial tonometry for coronary artery disease. Coron Artery Dis 2015;26:231–238
5. Rodriguez CJ, Miyake Y, Grahame-Clarke C, et al. Relation of plasma glucose and endothelial function in a population-based multiethnic sample of subjects without diabetes mellitus. Am J Cardiol 2005;96:1273–1277
6. Vehkavaara S, Seppälä-Lindroos A, Westerbacka J, Groop PH, Yki-Järvinen H. In vivo endothelial dysfunction characterizes patients with impaired fasting glucose. Diabetes Care 1999;22:2055–2060
7. Siasos G, Sara JD, Zaromytidou M, et al. Local low shear stress and endothelial dysfunction in patients with nonobstructive coronary atherosclerosis. J Am Coll Cardiol 2018;71:2092–2102
8. Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev Med 1995;46:223–234
9. Schmidt AM, Hori O, Chen JX, et al. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. J Clin Invest 1995;96:1395–1403
10. Vlassara H, Brownlee M, Manogue KR, Dinarello CA, Pasagian A. Cachectin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodeling. Science 1988;240:1546–1548
11. Muhlestein JB, Moreno FL. Coronary computed tomography angiography for screening in patients with diabetes: can enhanced detection of subclinical coronary atherosclerosis improve outcome? Curr Atheroscler Rep 2016;18:64
12. Liu HH, Cao YX, Li S, et al. Impacts of prediabetes mellitus alone or plus hypertension on the coronary severity and cardiovascular outcomes. Hypertension 2018;71:1039–1046
13. Caballero AE, Arora S, Saouaf R, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 1999;48:1856–1862
14. Park GM, Cho YR, Lee SW, et al. Prediabetes is not a risk factor for subclinical coronary atherosclerosis. Int J Cardiol 2017;243:479–484
15. Sara JD, Widmer RJ, Matsuzawa Y, Lennon RJ, Lerman LO, Lerman A. Prevalence of coronary microvascular dysfunction among patients with chest pain and nonobstructive coronary artery disease. JACC Cardiovasc Interv 2015;8:1445–1453

16. American Diabetes Association. Introduction: *Standards of Medical Care in Diabetes–2018*. Diabetes Care 2018;41(Suppl. 1):S1–S2
17. Marfella R, Sardu C, Calabrò P, et al. Non-ST-elevation myocardial infarction outcomes in patients with type 2 diabetes with non-obstructive coronary artery stenosis: effects of incretin treatment. Diabetes Obes Metab 2018;20:723–729
18. Marfella R, Sardu C, Balestrieri ML, et al. Effects of incretin treatment on cardiovascular outcomes in diabetic STEMI-patients with culprit obstructive and multivessel non obstructive-coronary-stenosis. Diabetol Metab Syndr 2018; 10:1
19. Westergren HU, Svedlund S, Momo RA, et al. Insulin resistance, endothelial function, angiogenic factors and clinical outcome in non-diabetic patients with chest pain without myocardial perfusion defects. Cardiovasc Diabetol 2016; 15:36
20. Lynch JJ, Ferro TJ, Blumenstock FA, Brockenauer AM, Malik AB. Increased endothelial albumin permeability mediated by protein kinase C activation. J Clin Invest 1990;85:1991–1998
21. Dresner A, Laurent D, Marcucci M, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest 1999;103:253–259
22. Wang XL, Zhang L, Youker K, et al. Free fatty acids inhibit insulin signaling-stimulated endothelial nitric oxide synthase activation through upregulating PTEN or inhibiting Akt kinase. Diabetes 2006;55:2301–2310
23. Griffin ME, Marcucci MJ, Cline GW, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 1999;48:1270–1274
24. Cosentino F, Eto M, De Paolis P, et al. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. Circulation 2003;107:1017–1023
25. Guzik TJ, Mussa S, Gastaldi D, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002;105:1656–1662
26. Inoguchi T, Li P, Umeda F, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000;49: 1939–1945
27. Inoguchi T, Sonta T, Tsubouchi H, et al. Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase. J Am Soc Nephrol 2003;14(Suppl. 3): S227–S232
28. Kruszelnicka O, Chyrchel B, Golay A, Surdacki A. Differential associations of circulating asymmetric dimethylarginine and cell adhesion molecules with metformin use in patients with type 2 diabetes mellitus and stable coronary artery disease. Amino Acids 2015;47:1951–1959
29. Tseng E, Yeh HC, Maruthur NM. Metformin use in prediabetes among U.S. adults, 2005-2012. Diabetes Care 2017;40:887–893